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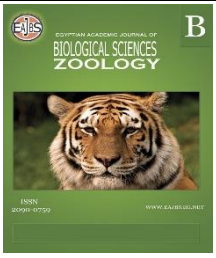

Assessment of Reproductive Toxicity of Silver Nanoparticles on Male Albino Mice "*Mus musculus*"

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ABSTRACT

Regarding the fast development of nanotechnology and its various applications, the present study was carried out to estimate the potential toxic effect of silver nanoparticles (AgNPs) on the fertility of male albino mice. Thirty-five adult male mice were randomly divided into 5 groups (7 mice per group) as the following: the first group (GP_1) was the control group which was orally administered distilled water. The second group (GP_2) and the third group (GP_3) were orally administrated silver nanoparticles (62.5 mg/kg/d & 125 mg/kg/d, respectively) for 35 days. The fourth group (GP_4) and the fifth group (GP_5) were orally administrated silver nanoparticles (62.5 mg/kg/d & 125 mg/kg/d, respectively) for 35 days then they were left 15 days without treatment for natural recovery and they were allowed to mate. Changes in the body or testicular weights or gonadosomatic index were recorded. Sperm analysis (sperm viability, motility, count and morphology) and damage in epididymal sperm DNA (comet assay) also were investigated. The results confirmed that silver nanoparticles have a toxic effect on male mice testis that leads to male infertility with high doses by reducing the number of sperms and increasing abnormalities in sperm motility and morphology.

INTRODUCTION

Nanoparticles are associated with modern science; they have a great scientific interest as they can be used in many fields of life. For example, in medicine, their ability to deliver drugs in the optimum dosage range often results in increased therapeutic efficiency of the drugs, weakened side effects and improved patient compliance (Alexis *et al.*, 2008). Also, foods and beverage products have been reported to contain NPs (Vance *et al.*, 2015). Among these nanoparticles silver nanoparticles (AgNPs) are widely used in a variety of applications as anti-bacterial agents in the health industry, food storage, textile coatings and a number of environmental applications where the cotton fibers containing AgNPs showed high anti-bacterial activity against *Escherichia coli* (Chen and Chiang, 2008). Also, AgNPs are widely used in consumer products, medical device coatings,

optical sensors, and cosmetics, in the pharmaceutical industry, the food industry, in diagnostics, orthopedics, and drug delivery, as anticancer agents, and have ultimately enhanced the tumor-killing effects of anticancer drugs. In addition, AgNPs have been frequently used in many textiles, keyboards, wound dressings, and biomedical devices (Gurunathan *et al.,* 2015) and in some disinfecting medical devices (Cho *et al.,* 2005; Jain and Pradeep, 2005; Li *et al.,* 2008).

Many studies investigated the effects of subchronic oral and inhalation toxicity of AgNPs in rodents (Kim et al., 2010; Park et al., 2010). In these studies, it was found that the accumulation of silver nanoparticles was observed in the blood and all tested organs, including the liver, spleen, kidneys, thymus, lungs, heart, brain, and testes. The problem comes from the highly oxidative activity of AgNPs which releases silver ions, these ions result in numerous negative effects on biological systems by inducing cytotoxicity, genotoxicity, immunological responses, and even cell death (Chernousova *et al.,* 2013; Cho *et al.,* 2013).

AgNPs can easily pass through the blood-brain barrier (BBB) by transcytosis of capillary endothelial cells or into other vital areas or tissues (Tang *et al.,* 2010). AgNPs have a bad effect on the cell cycle and can result in the induction of DNA hypermethylation which may have a negative effect on the epigenomic level (Mytych *et al.,* 2016). Also, AgNPs negatively affected male fertility because they have been found to reach the testes after administration (Kim *et al.,* 2010; Lee *et al.,* 2013). The negative effect of AgNPs appears when they reduce Glutathione (GSH) levels through the inhibition of GSH synthesizing enzyme (Piao *et al.,* 2011). GSH is considered one of the vital endogenous antioxidant scavengers that can bind to and reduce ROS. Thus GSH mediated antioxidant scavenge system is a critical defense system for cell survival (Dewanjee *et al.,* 2009). Through the inhibition of GSH synthesizing enzyme AgNPs raised intracellular ROS (Piao *et al.,* 2011). The increase in intracellular ROS activates cell death-regulating pathways by inducing apoptosis (Li *et al.,* 2016).

Silver nanoparticles negatively affect the male reproductive system. It can pass through the blood-testis barrier (Schrand *et al.,* 2010). A study on silver nanoparticles showed the effects of these NPs on buffalo sperm parameters, which showed a dose-dependent decrease in sperm viability without a change in sperm motility at a concentration of 50 mg/kg (Pothuraju *et al.,* 2013). Also, another study showed that silver nanoparticles can negatively affect sperm parameters. For example, it could damage sperm membranes and/or penetrate the cells, as a result increasing free radicals, including ROS, that cause membrane lipid peroxidation and, in consequence, loss of motility and viability, and can damage the sperm membrane and flagellum structure, finally leading to sperm motility and morphology defect (Yoshida *et al.,* 2004; Aziz *et al.,* 2004; Braydich-Stolle *et al.,* 2005). Additionally, Miresmaeili *et al.* (2013) showed a significant decrease in the number of primary spermatocyte and spermatid cells at doses of 50, 100 and 200 mg/kg. It has been indicated that AgNPs noticeably decreased spermatogonial stem cell proliferation by some intracellular pathways (Braydich-Stolle *et al.,* 2010).

Another study indicated that injecting Wistar mice with AgNPs via the tail vein resulted in an obvious decrease in sperm count. Not only the decrease in number but also more morphological changes were observed, folded, amorphous spermatozoa, cells lacking or showing a small hook, and cells with undulating or elongated heads were the most common abnormalities found (Gromadzka-Ostrowska, *et al.,* 2012; Lafuente, *et al.,* 2016). Moreover, exposure of murine sperm cells to AgNPs reduces the success rate of in vitro fertilization, delays following blastocyst formation, and down-regulates gene expression responsible for embryonic development (Yoisungnern *et al.,* 2015). Lee *et al.* recorded that AgNPs concentrations in the testes and brain did not return to the normal

control levels, even after the 4-month recovery period, showing that silver clearance is difficult across biological barriers, such as the BBB or BTB (Lee *et al.,* 2013). The present study aimed to evaluate the repro-toxic effects of silver nanoparticles on adult male mice *Mus musculus* and evaluate their natural recovery.

MATERIALS AND METHODS

Chemicals:

The material used in this research was silver nanoparticles (CAS Number: 7440-22-4, Sigma-Aldrich, USA). It was dissolved in distilled water. Silver nanoparticle, < 100 nm particle size, contains PVP as a dispersant, 99.5 % trace metals basis.

Experimental Animals:

Experimental animals used in this study were male mice (*Mus musculus*) approximately aged 8 weeks with an average weight of 28±3 gm animals were purchased from Vacsera, Egypt (VACSERA vivarium, Helwan, Egypt). Mice were acclimated to the lab environment for 7 days under normal conditions of lightning and ventilation. Animals were separated and housed in plastic cages with stainless steel mesh lids and given the standard diet and water ad-libitum during the experimental period.

Experimental Design:

Thirty-five mature male *Mus musculus* mice weighing 28±3 g were used in the present study. Under the same conditions, mice were randomly divided into 5 groups of 7 animals each. Animals of different experimental groups were exposed to oral doses of silver nanoparticles as follows:

<table>
<tr><th colspan="3" rowspan="2">Animal groups</th><th colspan="7">Duration of the experiment (weeks)</th></tr>
<tr><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th><th>7</th></tr>
<tr><td colspan="3">GP_1: Control</td><td colspan="5">Mice were provided with distilled water</td><td></td><td></td></tr>
<tr><td>GP_2</td><td rowspan="2">Ag NPs Treatment groups</td><td>62.5 mg/kg body weight of AgNPs</td><td colspan="5">Mice orally and daily treated with 62.5mg/kg/day</td><td></td><td></td></tr>
<tr><td>GP_3</td><td>125 mg/kg body weight of AgNPs</td><td colspan="5">Mice orally and daily treated with 125mg/kg/day</td><td></td><td></td></tr>
<tr><td>GP_4</td><td rowspan="2">15-day recovery groups</td><td>Low dose</td><td colspan="5">Mice orally and daily treated with 62.5mg/kg/day</td><td colspan="2">Left without treatment for recovery</td></tr>
<tr><td>GP_5</td><td>High dose</td><td colspan="5">Mice orally and daily treated with 125mg/kg/day</td><td colspan="2">Left without treatment for recovery</td></tr>
</table>

Mice in groups 1, 2 & 3 were sacrificed at the end of the 5th week whereas mice in groups 4&5 at the end of the 7th week. Animals of the control (GP1) and the first two treated groups (GP2, GP3) were orally treated for 35 days (a period of one spermatogenesis cycle in male albino mice (Clermont, 1972).

Reproductive Performance Study:

The fertility and the reproductive performance of both control and treated groups were studied at the end of treatment. Each male of the different studied groups was housed with a virgin untreated female 1:1 for 10 days (to complete two estrus cycles). Vaginal plugs were observed daily, the day on which the vaginal plug is detected is considered day zero of gestation. The mating index is calculated as the number of males mated and resulting in a vaginal plug over the number of males cohoused with females multiplied by 100 and expressed as the percent. The fertility index is expressed as the number of males

who sired a litter over the number of males resulting in a vaginal plug multiplied by 100 and expressed as a percentage (Hafez, 1970).

Evaluation of Body and Testes Weights and Gonadosomatic Index:

During the experimental period, the body weight of each animal was recorded weekly. The testis was dissected out, cleaned and weighed. The gonadosomatic index (GSI) was calculated by dividing the testis weight by the body weight of each animal and expressed as the percentage (Predes *et al.,* 2007).

Sperm Analysis:

The mice were sacrificed 24 h after the last dose. The left epididymis was removed and placed in a pre-warmed petri dish containing 0.1 ml of calcium and magnesium-free Hank's solution at 37˚C. The tissue was minced with scalpels for approximately 1 min. and placed in a 37˚C incubator for 15 min. The epididymis was processed for sperm motility, viability, count, and sperm abnormalities.

Sperm Motility:

The sperm motility was evaluated according to the method of Ekaluo *et al*. (Ekaluo *et al.,* 2013), two drops of sperm suspension were put on a microscope slide and covered with a cover slip and examined under the microscope at 40x magnification. The number of progressively motile sperm was recorded and divided by the total number of spermatozoa counted and expressed as a percentage.

Sperm Viability:

In order to determine sperm viability Eosin- Nigrosin stain method (Björndahl, *et al.,* 2003) was used. Equal volumes of sperm suspension and stain were mixed and on glass slides, smears were prepared and left to dry in the air before checking for viability then percentage viability was calculated by dividing the number of live sperm by the total number of sperm counted.

Sperm Count:

The epididymal sperm count was detected manually using the improved Neubauer hemocytometer by counting the heads under a light microscope. The count was recorded as the total number of sperm/ml (Ekaluo, *et al.,* 2008).

Sperm Abnormalities:

A volume of the sperm suspension was mixed with 1% eosin Y solution (10:1) for 30 min and smears were prepared on glass slides also were left to dry and then were examined for sperm (head, mid-piece and tail) abnormalities for every 200 spermatozoa per each slide. Abnormal sperm was calculated and expressed as the percentage according to Ekaluo *et al*. (Ekaluo, *et al.,* 2009).

Comet Assay:

The comet assay was performed to determine DNA damage as designated by (Singh, *et al.,* 1988) with minor modifications. Observations were made at 400X magnification using a fluorescent Z\xmicroscope (Olympus) equipped with an excitation filter of 515-560 nm and a barrier filter of 590 nm. When possible, fifty cells per animal were analyzed for DNA migration. The tail length was measured from the trailing edge of the nucleus to the leading edge of the tail, using a calibrated scale in the ocular. The severity of DNA damage was measured by comparing comet tail lengths (μm) with the diameter of the nucleus of undamaged cells observed in the same field.

Statistical Analysis:

Statistical analysis was performed using the analysis of variance (ANOVA) and Duncan's Multiple Range tests to determine differences among treatment means at a significant level of $p<0.05$ and highly significant at $p<0.01$, Standard errors were also estimated (Dytham, 1999). All statistics were run on the computer using SPSS program. All curves were fitted with the computer program office (2007). The paper has been

approved by the scientific and ethical committee of Zoology Department, Faculty of Science - Fayoum University 2009.

RESULTS

Firstly, the current study showed that silver nanoparticles have a lethal effect on mice treated with both high and low doses. The percent of mortality was 28.5% in GP_3, 14.2% in GP_4 and 42.9% in GP_5 while there was no mortality in GP_2.

Effect of Silver Nanoparticles Treatment on Body and Testis Weights and Gonadosomatic Index of Male Mice:

Table (1) showed that the body weight of the high dose (GP3) is high significantly decreased ($p<0.01$), (GP2) and (GP5) are significantly decreased ($p<0.05$) while (GP_4) is not significantly decreased compared to the control group. Meanwhile, the testis weight is significantly decreased ($p<0.05$) in GP_2, highly significantly decreased ($p<0.01$) in (GP_3, GP_5) and not significantly decreased in GP_4 when compared with the control group. Also, it is evident from this table that there is an improvement in testis weight and gonadosomatic index% in GP4 and GP5 but doesn't go back to normal levels in GP_5 when compared to the control group.

Table 1: Final body weight, total testes weights and gonadosomatic index in male albino mice treated with silver nanoparticles for 35 days (GP2 & GP3) and treated with silver nanoparticles for 35 days and another 15 days of natural recovery (GP4 & GP5).

Animal groups	Final body weight (g)	Total testes weight (g)	Gonadosomatic index %
Control GP1	31.59 ± 0.93 (A)	0.23 ± 0.02 (A)	0.72 ± 0.05 (A)
GP2	30.04 ± 0.88 Sig (B)	0.16 ± 0.02 Sig (B)	0.55 ± 0.05 Sig (B)
GP3	24.94 ± 0.82 Hi sig (C)	0.14 ± 0.02 Hi sig (C)	0.56 ± 0.06 Hi sig (C)
GP4	30.92 ± 0.92 Not sig (A)	0.22 ± 0.02 Not sig(A)	0.71 ± 0.06 Not sig (A)
GP5	30.19 ± 0.99 Sig (B)	0.20 ± 0.02 Hi sig (C)	0.67 ± 0.05 Sig(B)

Data are represented as the mean of samples ±SE.
Means with the same letter for each parameter in the same column is not significantly different, otherwise, they do (Duncan multiple range test).

Sperm Analysis:

Sperm Count:

The epididymal sperm concentration in mice showed a highly significant reduction ($p<0.01$) (23.28±2.8, 26.5±2.7 and 14.2±2.8×106/mlm3 in G2, G3 and G5 respectively, and significant reduction ($p<0.05$) (44.20±2.8 ×106/mlm3) in G4 compared to control group (69.2±2.6×106/mlm3) as shown in Table 2 and Figure 1A.

Sperm Motility:

Results showed that all treated groups with AgNPs showed a highly significant ($p<0.01$) decline in the percent of progressive motility (2.7±3.00, 14±2.8 and 10.4±2.8) GP_2, GP_3, and GP_5 respectively, compared to the control group (71.4±2.9) but GP_4 showed an improvement in the percent of progressive motility (51.2±3.00), significant ($p<0.05$) reduction, in comparison with GP_2 as shown in Table 2 and Figure 1B.

Sperm Viability:

Results showed that normal live sperms appeared whitish unstained, while dead sperms were stained and appeared pinkish (Fig. 1). It is obvious that low and high doses of AgNPs negatively affected the mice sperms and showed a highly significant decrease ($p<0.01$) in the sperm viability by a percent of (33.3±4.1%, 45±4.1% and 27.50±3.7%) GP_2, GP_3 and GP_5 respectively, in comparison with that of the control group (73.4±4.00%) but GP_4 showed a significant decrease ($p<0.05$) in the sperm viability by a percent of (61.7±4.2%) in comparison with that of the control group as shown in Table 2 and Figure 1C.

Table 2: Assessment of Sperm count ($X10^6/mm^3$), motility and viability of male albino mice treated with silver nanoparticles for 35 days (GP_2, GP_3) and treated with silver nanoparticles for 35 days and with another 15 days for probable natural recovery (GP_4 and GP_5).

Animal groups	Count × 10^6	Progressive Motility %	Viability %
Control GP_1	69.2 ± 2.6 (A)	71.4 ± 2.9 (A)	73.4 ± 4 (A)
GP_2	23.2 ± 2.8 High sig. (C)	2.7 ± 3 High sig. (C)	33.3 ± 4.1 High sig. (C)
GP_3	26.5 ± 2.7 High sig. (C)	14 ± 2.8 High sig. (C)	45 ± 4.1 High sig. (C)
GP_4	44.2 ± 2.8 High sig. (C)	51.2± 3 High sig. (C)	61.7 ± 4.2 High sig. (C)
GP_5	14.2 ± 2.8 High sig. (C)	10.4 ± 2.8 High sig. (C)	27.5 ± 3.7 High sig. (C)

Data are represented as mean of samples ±SE.
Means with the same letter for each parameter in the same column is not significantly different, otherwise, they do (Duncan multiple range test).

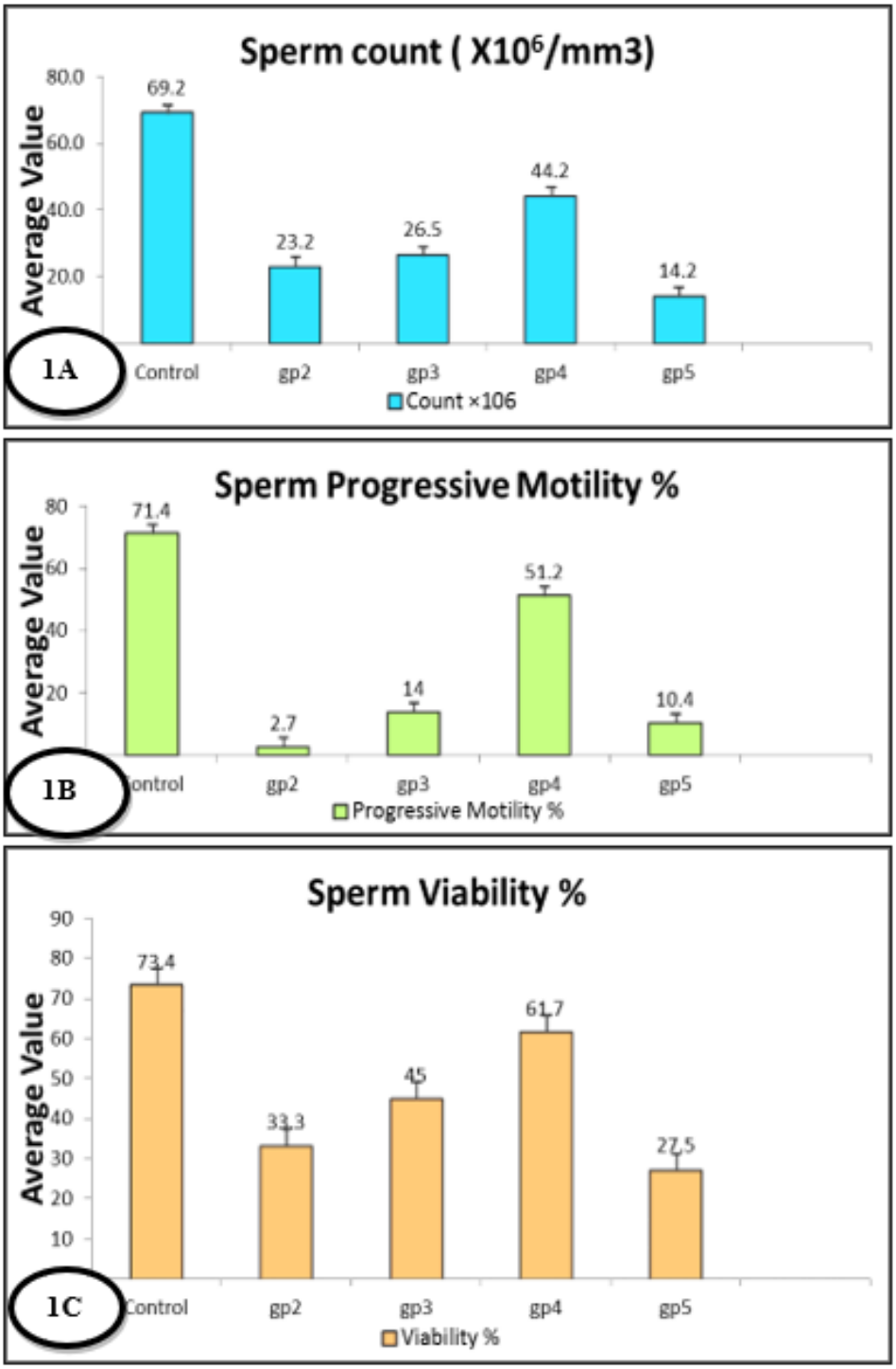
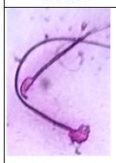


Fig. 1: A. Sperm count (X106/mm3) in male albino mice in different studied groups.
B. Sperm Progressive Motility% in male albino mice in different studied groups.
C. Sperm viability% in male albino mice in different studied groups**.**

Evaluation of Sperm Abnormalities:

The sperm abnormalities listed in (Table3) showed a highly significant increase in the incidence percent of sperm abnormalities in all silver nanoparticles treated groups compared with the control group.

Table 3: Types of the recorded sperm abnormalities in male albino mice in control and different treated with silver nanoparticles for 35 days (GP_2 and GP_3) and treated with silver nanoparticles for 35 days and another 15 days of natural recovery (GP_4 and GP_5).

Groups	Types and percent of sperm abnormalities								
	Percent of head abnormalities				Percent of mid-piece abnormalities		Percent of tail abnormalities		Percentage of total abnormalities
	Amorphous Head	Hookless	Hammer Shape	Double headed	Bent	Coiled	Doubled Tail	Coiled Tail	
									
GP_1	1.67 ± 0.58	0.33 ± 0.58	0.33 ± 0.58	0.33 ± 0.58	2.67 ± 1.53	3.67 ± 1.53	0.33 ± 0.58	7.00 ± 2.00	16.33 ± 7.94
GP_2	1.60 ± 0.71	1.20 ± 0.00	2.80 ± 0.71	0.20 ± 0.71	7.80 ± 1.22	7.60 ± 0.80	3.00 ± 0.82	19.80 ± 2.54	44.00 ± 7.49
GP_3	4.14 ± 0.82	7.0 ± 0.71	0.29 ± 0.71	0.43 ± 0.71	12.00 ± 2.04	10.43 ± 1.53	0.86 ± 0.00	16.14 ± 1.41	51.29 ± 7.92
GP_4	0.25 ± 0.71	10.75 ± 0.53	0.50 ±0.00	0.25 ± 0.00	10.75 ± 2.04	12.75 ± 1.63	0.50 ± 0.71	20.75 ± 0.53	56.50 ± 6.16
GP_5	6.00 ± 0.00	3.67 ± 0.41	0.67 ± 0.41	0.50 ± 0.00	4.50 ± 0.80	5.00 ± 2.24	1.33 ± 0.71	9.83 ± 0.23	31.50 ± 4.80

Data are represented as mean of samples ±SE. Means with the same letter for each parameter in the same column is not significantly different, otherwise, they do (Duncan multiple range test).

Comet Assay:

Comet assay showed that silver nanoparticles stimulated statistically highly significant ($P<0.01$) increase in the average of the comet % from (8.26) in the control group to (12.97) in GP2, (17.75) in GP3, (15.75) in GP_5 and not significant increase (8.79) in GP_4. Tail length was significantly increased ($p<0.05$) by 3.87 in GP_2 and 3.93 in GP5 compared to the control group (2.86), a highly significant increase ($p<0.01$) by 6.20 in GP3 and there was no significant increase by 3.59 in GP_4 when compared to the control group (2.86). The percent of DNA in comet tail showed a highly significant increase ($p<0.01$) (27.19 in GP_2, 30.62 in GP3, 27.51 in GP_5) and there was no significant increase (17.65 in GP4) compared to the control group (15.42). As well tail moment showed a highly significant increase ($p<0.01$) (1.13 in GP_2, 1.42 in GP_3 and 1.16 in GP_5) and there was no significant increase (0.75 in GP_4) compared to the control group (0.62).

Table 4: Comet assay in the sperm of male albino mice treated with silver nanoparticles for 35 days (GP2 & GP3) and treated with silver nanoparticles for 35 days and another 15 days of natural recovery (GP4 & GP5).

Animal groups	Comet %	Tail length	% DNA in tail	Tail Moment
Control GP1	8.26 ± 0.22 (A)	2.86 ± 0.15 (A)	15.42 ± 0.78 (A)	0.62 ± 0.06 (A)
GP2	12.97 ± 0.23 High sig. (C)	3.87 ± 0.23 Sig. (B)	27.19 ± 0.23 High sig. (C)	1.13 ± 0.21 High sig. (C)
GP3	17.75 ± 0.24 High sig. (C)	6.20 ± 0.18 High sig. (C)	30.62 ± 0.23 Hi sig (C)	1.42 ± 0.24 High sig. (C)
GP4	8.79 ± 0.23 Not sig. (A)	3.59 ± 0.23 Not sig. (A)	17.65 ± 0.24 Not sig (A)	0.75 ± 0.24 Not sig. (A)
GP5	15.75 ± 0.24 High sig. (C)	3.93 ± 0.23 Sig. (B)	27.51 ± 0.24 High sig. (C)	1.16 ± 0.24 High sig. (C)

Data are represented as mean of samples ±SE
Means with the same letter for each parameter in the same column is not significantly different, otherwise, they do (Duncan multiple range test).

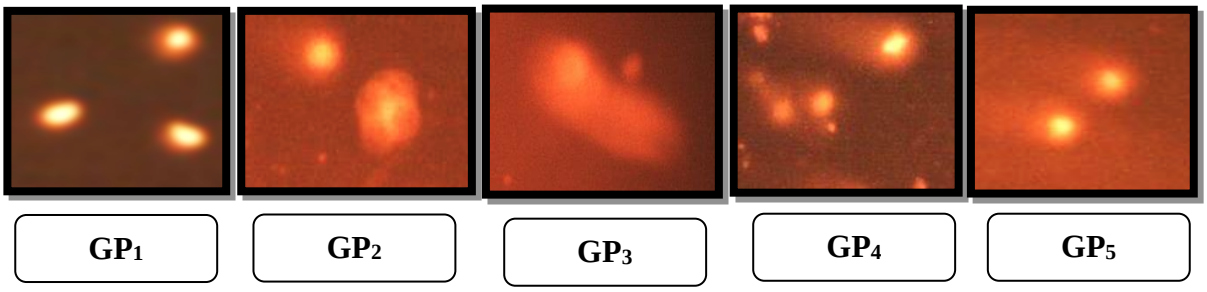


Fig. 2: Photographs of comet assay of sperms showing DNA damage induced in the sperms of control male albino mice and different treated AgNPs groups.

DISCUSSION

In the present study, mice treated with silver nanoparticles 62.5mg/kg and 125 mg/kg body weight (GP_2 and GP_3), respectively for 35 days showed significant and highly significant decreases, respectively in their body weights in a dose-dependent manner compared with the control group, Also mice treated with 125 mg/kg body weight plus 15 days of natural recovery (GP_5) showed a significant decrease in their body weights compared with the control group while mice treated with 62.5 mg/kg body weight plus 15 days of natural recovery (GP_4) showed no significant decrease in their body weights compared with the control group. These results agree with the studies done by Shahare *et al.* who recorded that oral exposure of 5–20 nm silver nanoparticles in mice for 21 days to 5 mg/kg/day of body weight resulted in a reduction in body weight (Shahare, *et al.,* 2013) and also with another study of 13-week oral administration of 500 mg/kg/day of 60 nm silver nanoparticles of body weight in rats resulted in a decline in the body weight of male rats only (Kim *et al.,* 2010). This decline in weight may be due to the overproduction of ROS. According to Son, *et al.* (2015) and Rocca, *et al.* (2004), the disturbance in ROS homeostasis has been shown to be one of the major mechanisms through which nanoparticles cause toxicity to various physiological processes including white adipocytes functionality. Braydich-Strolle *et al.* (2010) used mouse stem cells and recorded that smaller silver nanoparticles are more expected to produce ROS and cause apoptosis. From the present and previous studies, AgNPs could cause weight loss by stimulating the production of ROS.

In the current study, we recorded a significant decline in the testis weight in GP_2, a highly significant decrease in GP_3 and GP_5 while there was no significant decrease in GP_4 when compared to the control group. While gonadosomatic index showed a significant decrease in GP_2 and GP_5, a highly significant decrease in GP_3 and there no significant decrease in GP_4 when compared to the control group. So, GP_4 showed an improvement in their body weight, testis weight and gonadosomatic index. The present study showed a significant increase in sperm abnormalities including, sperm motility, viability, count and morphology. This agreed with Gromadzka-Ostrowskaa *et al*. (2012) who reported that a size-dependent (20 nm and 200 nm), dose-dependent (5 and 10 mg/kg body mass) and time-dependent (24 h, 7 and 28 days) decrease in the epididymal sperm count in rats and an increase in a number of dead sperms after treatment with AgNPs. Also, Kruszewski *et al*. showed that AgNPs could react with cellular DNA and induce inflammation, oxidative damage and cellular dysfunction that resulted in genetic mutation and sperm cells with abnormal morphology (Kruszewski *et al*., 2011).

In another study, Miresmaeili *et al.* (2013) reported a significant decline in the mean number of primary spermatocytes, spermatids and sperm cells and backed this effect

to the inhibitory role of AgNPs on cell proliferation. Also, Terzuoli *et al.* (2011) reported that increasing concentrations of AgNPs significantly reduced human sperm motility. As mentioned previously, AgNPs can induce ROS production and according to Aziz *et al.* (2004), ROS level is positively correlated with the proportion of sperm with amorphous heads, damaged acrosomes, midpiece defects, cytoplasmic droplets and tail abnormalities. In another study, Nel *et al.* (2006) demonstrated that AgNPs may raise the rates of abnormalities in sperm morphology and genetic mutations. Again, Baki *et al.* (2014) recorded a significant decline in sperm normal morphology of AgNPs treated animals which were dependent on the dose of these particles. Parallel with these results Mangelsdorf *et al.* (2003) revealed that a decrease in the total sperm count, increase in abnormal sperm morphology, impairment in the stability of sperm chromatin, or damage in sperm DNA leads to the disruption of spermatogenesis at any stage of cell differentiation. In addition, Takeda *et al.* (2009) reported that the damaged seminiferous tubules caused by a high dose of AgNPs may be related to the inhibitory role of these particles in cell proliferation, on cell cycle and the significant decline in sperm precursor cells or the release of them to the mid duct of seminiferous tubules. As well the study of Hofmann *et al.* (2005) showed that AgNPs interfere with spermatogonial stem cell proliferation in a dose-dependent and particle size-dependent manner. These abnormal effects were consistent with the results of the present study.

Furthermore, Garcia *et al.* (2014) reported that sub-acute (short-term) intravenous administration of AgNPs in male mice could be toxic to the male reproductive system and altered Leydig cell function and testosterone levels. Braydich-Stolet *et al*. (2005) revealed that silver nanoparticles could cross the sperm membrane and connect to mitochondria and the acrosome of sperm cells. Also, McAuliffe *et al.* (2007) showed that nanoparticles can cross cell membranes easily and even move across the blood-brain barrier and blood-testes barrier. Similar results were presented in rats by Miresmaeili *et al.* (2013).

Considering the genotoxic effect of AgNPs, comet assay is more efficient due to its sensitivity for detecting low levels of DNA damage. The present study showed clear DNA degradation in the nuclei of sperms resulting in an increase in the percentage of tail DNA in comparison with the control group. In this respect, various studies showed that AgNPs can stimulate oxidative stress and ROS generation (Wang *et al.*, 2017). Also, Asharani *et al.* (2008) suggested that AgNPs can stimulate disruption of the mitochondrial respiratory chain, as a result, increased ROS production and disruption of ATP synthesis, and finally resulting in DNA damage. El-Tohamy, (2012) found that the overproduction of ROS can be detrimental to the produced sperms as it is may have resulted in male infertility. This may be due to the effect of ROS on sperm DNA, as recorded by (Twigg *et al*., 1998) who reported that ROS affects the sperm genome, causing high frequencies of single and double-strand DNA breaks. They are also known to cause lipid peroxidation of sperm plasma membranes, resulting in the alteration of sperm function and fertilizing capacity (Duru *et al*., 2000). Moreover, Aitken and De Iuliis, (2009) showed that any damage to the sperm DNA increases the possibility of infertility, miscarriage, or serious disease in the offspring.

CONCLUSION:

The results of the present study showed that silver nanoparticle exposure could stimulate toxicological effects on the testicular tissues, and spermatogenic process and affect sperm parameters negatively with potential risks and possible consequences on fertility and reproduction.

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